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Imprinting of Confining Sites for Cell Cultures on Thermoplastic Substrates

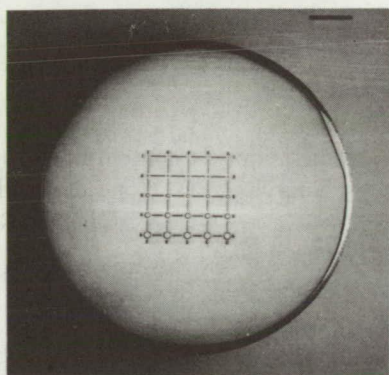


FIGURE 1. Die face etched with a pattern of lagoons (ranging in diameter from 30 to 965 μ) and coordinates. Scale bar, 0.25 inch.

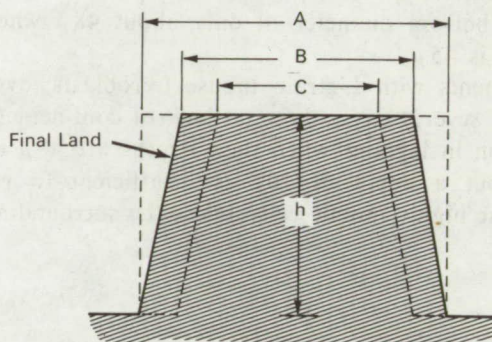


FIGURE 2. Compensation for reduction of lagoon's diameter during etching. A, Diameter of photographic image required to effect a final land diameter equal to B; B, final diameter of land; C, final diameter of land if the diameter of photographic image equaled B.

Because cells, cultured either singly or in small groups in monolayers, are often highly mobile, it is frequently necessary to prevent migration of test cells beyond the field of observation, or mixing with neighboring cultures. Cells or cultures can be so confined by growth in tiny ponds or microlagoons made in either a layer of grease (1) or a thermoplastic substrate. Thermoplastic films or dishes (such as Petri dishes) are easily imprinted with specifically designed patterns of microlagoons in a manner now to be described; the range of possible patterns, sizes, shapes, and depths of lagoons is infinite, but one typical example—for impression of patterns on the bottoms of 70-mm Petri dishes—will serve to explain the method.

To the bottom of a brass die blank (2 inches in diameter, 5/8-inch thick, and having a hole in its side to accommodate a soldering iron) is soldered a 2-inch disk of 0.030-inch-thick beryllium copper. The copper

surface is then polished to a mirror finish (4 micro-inches) before it is degreased and cleaned. The desired pattern of lagoons (Fig. 1), which range in diameter from 30 to 1,000 μ , is drawn on paper at a scale of 20 \times and photographed on high-contrast positive film at 20:1 reduction. Under partial vacuum (5 to 7 mm-Hg absolute) the image is then contact-printed on the die's polished surface which has been coated with a photographic emulsion sensitive to ultraviolet light and resistant to FeCl_3 . The die is then developed.

In a spray chamber the die's surface is then etched with FeCl_3 to a depth of 45 μ —the required depth of lagoon; copper is removed at the rate of about 30.5 μ /min at 100°F. After removal of the remnant emulsion the die is ready to imprint.

For imprinting, the die is placed face upward on a sheet of thermal insulator in the center of the base plate of a drill press or milling machine on the spindle

(continued overleaf)

of which is mounted a polished pressure plate. Die and pressure plate are heated with soldering irons each regulated by a rheostat to a temperature of 180°F for Falcon culture ware; 120°F for poly(vinyl chloride) ware. The soldering irons are then removed, and the dish or blank to be imprinted is laid over the die for 15 seconds (for evaporation of surface moisture) before the pressure plate is pressed down for about 20 seconds. The imprinted vessel is then ready for use.

Since the etchant eats the sides of an image spike as well as the face of the die, the sides of the spike form an angle of about 15° from the vertical; the result is that the bottom areas of the lagoons in the finished plastics are somewhat smaller than the designed areas. This effect can be compensated by oversizing in the initial photographic pattern (Fig. 2). For example, a design diameter of 100 μ results in a plastic lagoon having a bottom diameter of only about 48 μ when the depth is 45 μ .

Experiments with L-strain mouse fibroblasts, over periods of several days, show no lack of confinement or isolation in lagoons when their depths are 40 μ or greater, but a depth of 10 μ is insufficient to restrain these highly mobile cells unless the surrounding

concentrations of cells are very sparse. The prime factors in prevention of escape of cells and intrusion, respectively, seem to be the sharpness of the angle between the lagoon's bottom and walls, and the relative sharpness of the angle at the top of the walls. Lagoons may be shaped at will, and their overall pattern may be matched with the field of the microscope.

Reference:

1. C. D. Cone, Jr., *Exp. Cell Res.* **48**, 212 (1967);— and K. H. Peddrew, *NASA Tech. Note TN D-4906* (Langley Research Center, 1968).

Notes:

1. This process is of interest to microbiologists.
2. Inquiries regarding this process may be addressed to:

Langley Research Center
Langley Station
Hampton, Virginia 23365
Reference: B69-10236

Patent status:

No patent action is contemplated by NASA.

Source: C. D. Cone, Jr., and E. N. Fleenor
Langley Research Center
(LAR-10495)